PCT/AU03/01588

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I, JONNE YABSLEY, ACTING TEAM LEADER EXAMINATION SUPPORT AND SALES hereby certify that annexed is a true copy of the Provisional specification in connection with Application No. 2002953095 for a patent by BIOTA SCIENTIFIC MANAGEMENT PTY LTD as filed on 29 November 2002.

WITNESS my hand this
Sixteenth day of December 2003

J R Yabsley

JONNE YABSLEY
ACTING TEAM LEADER
EXAMINATION SUPPORT AND SALES

PRIORITY DOCUMENT
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AUSTRALIA

Patents Act 1990

PROVISIONAL SPECIFICATION

Applicant(s):

BIOTA SCIENTIFIC MANAGEMENT PTY LTD

A.C.N. 006 477 710

Invention Title:

NOVEL CHEMICAL COMPOUNDS AND THEIR USE

The invention is described in the following statement:

NOVEL CHEMICAL COMPOUNDS AND THEIR USE

The present invention relates to new chemical compounds and their use in medicine. In particular the invention concerns novel prodrugs of pharmaceutical moieties, more specifically antimicrobial agents, methods for their preparation, pharmaceutical formulations containing them and their use in the treatment of microbial infections.

BACKGROUND OF THE INVENTION

The use of prodrugs as progenitors of pharmaceutical moieties is widespread and there are numerous examples of prodrug therapeutics that are converted to active drugs *in vivo*. Such prodrugs may be designed to improve absorption of pharmaceutical agents by, for example, the gastrointestinal tract.[1] Prodrugs may also be produced to facilitate transport across the blood-brain barrier,[2] provide slow release of pharmaceutically active agents,[3] improve patient acceptance[4] and minimise side effects.[5]

We have now developed prodrugs of pharmaceutical moieties, more specifically antimicrobial agents. These prodrugs are designed with the specific purpose of increasing residency time at epithelial surfaces such as the lung and airways, urinary and gastrointestinal tracts, blood vessels and skin. To accomplish this, the pharmaceutical moieties are converted to prodrugs by modification with a pharmacokinetic regulator by way of a linker group that can be cleaved *in vivo* to expose the drug.

It is envisaged that interaction with cell membranes will also increase residence times in major organs such as the liver and central nervous system given appropriate routes of administration. Organ specific targeting groups (labile or otherwise) could also be attached to the prodrug to enhance the delivery process.

SUMMARY OF THE INVENTION

In a first aspect, the present invention provides a prodrug of general formula (I):

X - L - Y

(I)

in which

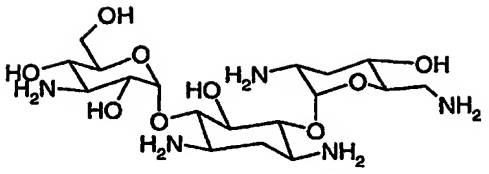
X is a pharmaceutically active moiety;

L is a linker group; and

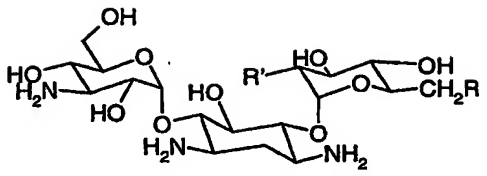
Y is a pharmacokinetic regulator,

or a pharmaceutically acceptable derivative or salt thereof.

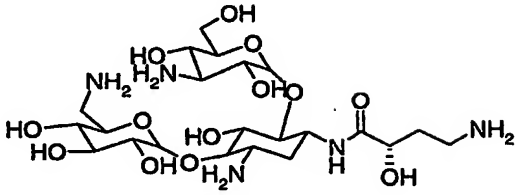
Preferably, the pharmaceutically active moiety is an antibacterial agent such as the following aminoglycosides:



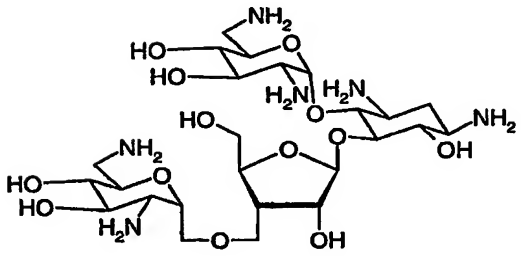
Tobramycin



	R	R'
Kanamycin A	NH_2	OH
Kanamycin B	NH_2	NH_2
Kanamycin C	OH	NH_2

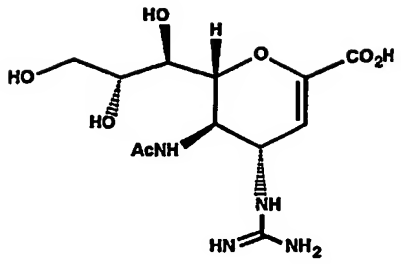


Amikacin



Neomycin

beta-lactam antibiotics, vancomycin and ciprofloxacin; an antiviral agent, for example, nucleosides, rhinovirus capsid-binding compounds, antisense oligonucleotides, peptides, inhibitors of HIVRT and inhibitors of influenza neuraminidase, for example, a compound of formula (A)



Compound (A)
Ac represents acetyl

; an antifungal agent such as amphotericin β or azoles, for example, fluconazole or ketaconazole; or an antiparasitic agent such as aspartic proteinases.

In a second aspect, the present invention provides a method for the preparation of the prodrug of formula (I) as defined above which comprises the steps of:

(a) optionally protecting the pharmaceutically active moiety X and/or the linker group which is attached to the optionally protected pharmacokinetic regulator Y;

(b) reacting the optionally protected pharmaceutically active moiety X and the optionally protected linker group L attached to the optionally protected pharmacokinetic regulator Y; and

(c) if necessary, removing the protecting groups of the pharmaceutically active moiety X, the linker L and the pharmacokinetic regulator Y.

In a third aspect, the invention provides the prodrug of formula (I) or a pharmaceutically acceptable derivative thereof, for use as an active therapeutic agent in the treatment of a microbial infection.

A fourth aspect of the invention provides a method for the prevention or treatment of a microbial infection comprising the step of administering to a subject in need thereof an effective amount of the prodrug of formula (I) or a pharmaceutically acceptable salt or derivative thereof.

In a fifth aspect, the invention provides use of the prodrug of formula (I) for the manufacture of a medicament for the treatment of a microbial infection.

In a sixth aspect, the invention provides a method for the detection of a microbial infection which comprises the step of contacting the prodrug of formula (I) with a sample suspected of containing the microorganism.

In a seventh aspect, the invention provides a pharmaceutical formulation comprising the compound of formula (I) or a pharmaceutically acceptable salt or derivative thereof, together with one or more pharmaceutically acceptable carriers and, optionally, other therapeutic and/or prophylactic ingredients.

According to an eighth aspect of the present invention there is provided an inhaler which contains the formulation as defined above.

DETAILED DESCRIPTION OF THE INVENTION

For the purposes of this specification it will be clearly understood that the word "comprising" means "including but not limited to", and that the word "comprises" has a corresponding meaning.

The pharmaceutically-active moiety X may be selected from synthetic or natural peptides, proteins, mono- or oligosaccharides, sugar-amino acid conjugates, sugar-peptide conjugates, toxins, drugs, pro-drugs or drug like molecules. Also included for moiety X are antibodies or antigen binding fragments of whole antibody, wherein the fragments retain the binding specificity of the whole antibody molecule. The binding fragments include, for example, Fab, F(ab')2, and Fv fragments. Binding fragments can be obtained using conventional techniques, such as proteolytic digestion of antibody by papsin or pepsin, or through standard genetic engineering techniques that are known in the art.

Indeed, the present invention is intended to encompass and be suitable for any pharmaceutically active moiety, especially any of the following drugs:

1. Analgesic anti-inflammatory agents such as, acetaminophen, aspirin, salicylic acid, methyl salicylate, choline salicylate, glycol salicylate, l-menthol, camphor, mefanamic acid, fluphenamic acid, indomethacin, diclofenac, alclofenac, ibuprofen, ketoprofen, naproxene, pranoprofen, fenoprofen, sulindac, fenbufen, clidanac, flurbiprofen, indoprofen, protizidic acid, fentiazac, tolmetin, tiaprofenic acid, bendazac, bufexamac, piroxicam, phenylbutazone, oxyphenbutazone, clofezone, pentazocine, mepirizole and the like;

2. Drugs having an action on the central nervous system, for example sedatives, hypnotics, antianxiety agents, anticholinesterase agents, analgesics and anesthetics, such as, chloral, buprenorphine, naloxone, haloperidol, fluphenazine, pentobarbital, phenobarbital, secobarbital, amobarbital, cydobarbital, codeine, lidocaine, tetracaine, dyclonine, dibucaine, cocaine, procaine, mepivocaine, bupivacaine, etidocaine, prilocaine, benzocaine, fentanyl, nicotine, galanthamine and the like;

3. Antihistaminics or antiallergic agents such as, diphenhydramine, dimenhydrinate, perphenazine, triprolidine, pyrilamine, chlorcyclizine, promethazine, carbinoxamine, tripelennamine, brompheniramine, hydroxyzine, cyclizine, meclizine, cloprenaline, terfenadine, chlorpheniramine and the like;

4. Acetonide anti-inflammatory agents, such as hydrocortisone, cortisone, dexamethasone, fluocinolone, triamcinolone, medrysone, prednisolone, flurandrenolide, prednisone, halcinonide, methylprednisolone, fludrocortisone, corticosterone, paramethasone, betamethasone, ibuprophen, naproxen, fenoprofen, fenbufen, flurbiprofen, indoprofen, ketoprofen, suprofen, indomethacin, piroxicam, aspirin, salicylic acid, diflunisal, methyl salicylate, phenylbutazone, sulindac, mefenamic acid, meclofenamate sodium, tolmetin and the like;

5. Steroids such as, androgenic steroids, for example, testosterone, methyltestosterone, fluoxymesterone, estrogens for example, conjugated estrogens, esterified

estrogens, estropipate, 17β-estradiol, 17β-estradiol esters such as 17β-estradiol valerate, equilin, mestranol, estrone, estriol, 17β-estradiol derivatives such as 17β-ethinyl estradiol, diesthylstilbestrol, progestational agents, such as, progesterone, 19-norprogesterone, norethindrone, norethindrone acetate, melengestrol, chlormadinone, ethisterone, medroxyprogesterone acetate, hydroxyprogesterone caproate, ethynodiol diacetate, norethynodrel, 17α-hydroxyprogesterone, dydrogesterone, dimethisterone, ethinylestrenol, norgestrel, demegestone, promegestone, megestrol acetate and the like;

6. Respiratory agents such as, theophylline and β_2-adrenergic agonists, for example, albuterol, terbutaline, metaproterenol, ritodrine, carbuterol, fenoterol, quinterenol, rimiterol, solmefamol, soterenol, tetroquinol and the like;

7. Sympathomimetics such as, dopamine, norepinephrine, penylpropanolamine, pheylephrine, psuedoephedrine, amphetamine, propylhexedrine, arecoline and the like;

8. Antimicrobial or antiinfective agents including antibacterial agents, antifungal agents, antiparasitic agents, antimycotic agents and antiviral agents, such as, those listed in the Ashgate Handbook of Anti-Infective Agents (Ed G.W.A. Milne, Ashgate Publishing, 2000), for example, tetracyclines such as oxytetracycline; penicillins such as ampicillin; cephalosporins such as cefalotin; aminoglycosides such as kanamycin, amikacin, neomycin and tobramycin; macrolides such as erythromycin, chloramphenicol, iodides, nitrofrantoin; antifungals such as clotrimazole, miconazole, chloramphenicol, nystatin, amphotericin, fradiomycin, sulfonamides, purrolnitrin, sulfacetamide, sulfamethazine, sulfadiazine, sulfamerazine, sulfamethizole and sulfisoxazole; antivirals such as inhibitors of influenza neuraminidase and idoxuridin; clarithromycin; and other anti-infectives including nitrofurazone and the like;

9. Antihypertensive agents such as, clonidine, α-methyldopa, reserpine, syrosingopine, rescinnamine, cinnarizine, hydrazine, prazosin and the like;

10. Antihypertensive diuretics such as, chlorothiazide, hydrochlorothrazide, bendoflumethazide, trichlormethiazide, furosemide, tripamide, methylclothiazide, penfluzide, hydrothiazide, spironolactone, metolazone and the like;

11. Cardiotonics such as, digitalis, ubidecarenone, dopamine and the like;

12. Coronary vasodilators such as, organic nitrates such as, nitroglycerine, isosorbitol dinitrate, erythritol tetranitrate, and pentaerythritol tetranitrate, dipyridamole, dilazep, trapidil, trimetazidine and the like;

13. Vasoconstrictors such as, dihydroergotamine, dihydroergotoxine and the like;

14. β-blockers or antiarrhythmic agents such as, timolol pindolol, propranolol and the like;

15. Calcium antagonists and other circulatory organ agents, such as, aptopril, diltiazem, nifedipine, nicardipine, verapamil, bencyclane, ifenprodil tartarate, molsidomine, clonidine, prazosin and the like;

16. Anti-convulsants such as, nitrazepam, meprobamate, phenytoin and the like;

17. Agents for dizziness such as, isoprenaline, betahistine, scopolamine and the like;

18. Tranquilizers such as, reserprine, chlorpromazine, and antianxiety benzodiazepines such as, alprazolam, chlordiazepoxide, clorazeptate, halazepam, oxazepam, prazepam, clonazepam, flurazepam, triazolam, lorazepam, diazepam and the like;

19. Antipsychotics such as, phenothiazines including thiopropazate, chlorpromazine, triflupromazine, mesoridazine, piperracetazine, thioridazine, acetophenazine, fluphenazine, perphenazine, trifluoperazine, and other major tranquilizers such as, chlorprathixene, thiothixene, haloperidol, bromperidol, loxapine, and molindone, as well as, those agents used at

lower doses in the treatment of nausea, vomiting and the like;

20. Muscle relaxants such as, tolperisone, baclofen, dantrolene sodium, cyclobenzaprine and the like;

21. Drugs for Parkinson's disease, spasticity, and acute muscle spasms such as levodopa, carbidopa, amantadine, apomorphine, bromocriptin, selegiline (deprenyl), trihexyphenidyl hydrochloride, benztropine mesylate, procyclidine hydrochloride, baclofen, diazepam, dantrolene and the like;

22. Respiratory agents such as, codeine, ephedrine, isoproterenol, dextromethorphan, orciprenaline, ipratropium bromide, cromglycic acid and the like;

23. Non-steroidal hormones or antihormones such as, corticotropin, oxytocin, vasopressin, salivary hormone, thyroid hormone, adrenal hormone, kallikrein, insulin, oxendolone and the like;

24. Vitamins such as, vitamins A, B, C, D, E and K and derivatives thereof, calciferols, mecobalamin, and the like for dermatological use;

25. Antitumor agents such as, 5-fluorouracil and derivatives thereof, krestin, picibanil, ancitabine, cytarabine and the like;

26. Enzymes such as, lysozyme, urokinaze and the like;

27. Herb medicines or crude extracts such as, glycyrrhiza, aloe, Sikon (Lithospermi radix) and the like;

28. Miotics such as pilocarpine and the like;

29. Cholinergic agonists such as, choline, acetylcholine, methacholine, carbachol, bethanechol, pilocarpine, muscarine, arecoline and the like;

30. Antimuscarinic or muscarinic cholinergic blocking agents such as, atropine, scopolamine, homatropine, methscopolamine, homatropine methylbromide, methantheline, cyclopentolate, tropicamide, propantheline, anisotropine, dicyclomine, eycatropine and the like;

31. Mydriatics such as, atropine, cyclopentolate, homatropine, scopolamine, tropicamide, eucatropine, hydroxyamphetamine and the like;

32. Psychic energizers such as, 3-(2-aminopropyl)indole, 3-(2-aminobutyl)indole and the like;

33. Humoral agents such as, the prostaglandins, natural and synthetic, for example, PGE_1, $PGE_{2\alpha}$, and $PGF_{2\alpha}$, and the PGE_1 analog misoprostol.

34. Antispasmodics such as, atropine, methantheline, papaverine, cinnamedrine, methscopolamine and the like;

35. Antidepressant drugs such as, isocarboxazid, phenelzine, tranylcypromine, imipramine, amitriptyline, trimipramine, doxepin, desipramine, nortriptyline, proptriptyline, amoxapine, maprotiline, trazodone and the like;

36. Anti-diabetics such as, insulin, and anticancer drugs such as, tamoxifen, methotrexate and the like;

37. Anorectic drugs such as, dextroamphetamine, methamphetamine, phenylpropanolamin, fenfluramine, diethylpropion, mazindol, phentermine and the like;

38. Anti-allergenics such as, antazoline, methapyrilene, chlorpheniramine, pyrilamine, pheniramine and the like;

39. Decongestants such as, phenylephrine, ephedrine, naphazoline, tetrahydrozoline and the like;

40. Antipyretics such as, aspirin, salicylamide and the like;

41. Antimigrane agents such as, dihydroergotamine, pizotyline and the like;

42. Anti-malarials such as, the 4-aminoquinolines, alphaaminoquinolines, chloroquine, pyrimethamine and the like;

43. Anti-ulcer agents such as, misoprostol, omeprazole, enprostil, allantoin, aldioxa, alcloxa, N-methylscopolamine methylsulfate and the like;

44. Peptides such as, growth releasing factor and the like;

45. Anti-estrogen or anti-hormone agents such as, tamoxifen or human chorionic gonadotropin and the like.

The term "linker group" is used herein in its broadest sense and refers to a functional group capable of being cleaved *in vivo* to expose the pharmaceutical moiety X. Suitable linker groups include esters, amides, ureas, thioureas, imines, acetals, ethers, phosphates, phosphate esters or diesters, thioesters, oximes and hydrazones. Preferably the linker group is an ester, amide or phosphate ester, more preferably an ester.

The term "pharmacokinetic regulator" is used herein in its broadest sense and refers to a moiety which is capable of regulating residency time and the intensity of release of the pharmaceutical moiety X. The pharmacokinetic regulator may be a hydrophobic or hydrophilic moiety.

Suitable hydrophobic moieties include straight chain, branched and cyclic hydrocarbons such as alkyl having 2 to 24 carbon atoms, preferably 2 to 16 carbon atoms, more preferably 2 to 10 carbon atoms, most preferably 2 to 6 carbon atoms. Illustrative of straight chain and branched alkyl are ethyl, propyl, isopropyl, butyl, isobutyl, sec-butyl, tert-butyl, amyl, isoamyl, sec-amyl, 1,2-dimethylpropyl, 1,1-dimethylpropyl, hexyl, 4-methylpentyl, 1-methylpentyl, 2-methylpentyl, 3-methylpentyl, 1,1-dimethylbutyl, 2,2-dimethylbutyl, 3,3-dimethylbutyl, 1,2-dimethylbutyl, 1,3-dimethylbutyl, 1,2,2-trimethylpropyl, 1,1,2-trimethylpropyl, heptyl, 5-methylhexyl, 1-methylhexyl, 2,2-dimethylpentyl, 3,3-dimethylpentyl, 4,4-dimethylpentyl, 1,2-dimethylpentyl, 1,3-dimethylpentyl, 1,4-dimethylpentyl, 1,2,3-trimethylbutyl, 1,1,2-trimethylbutyl, 1,1,3-trimethylbutyl, octyl, 6-methylheptyl, 1-methylheptyl, 1,1,3,3-tetramethylbutyl, nonyl, 1-, 2-, 3-, 4-. 5-, 6- or 7-methyloctyl, 1-, 2-, 3-, 4- or 5-ethylheptyl, 1-, 2- or 3-propylhexyl, decyl, 1-, 2-, 3-, 4-, 5-, 6-, 7- and 8-methylnonyl, 1-, 2-, 3-, 4-, 5- or 6-ethyloctyl, 1-, 2-, 3- or 4-propylheptyl, undecyl, 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8- or 9-methyldecyl, 1-, 2-, 3-, 4-, 5-, 6- or 7-ethylnonyl, 1-, 2-, 3-, 4- or 5-propyloctyl, 1-, 2- or 3-butylheptyl, 1-pentylhexyl, dodecyl, 1-, 2-, 3-, 4-, 5-, 6-, 7-, 8-, 9-

or 10-methylundecyl, 1-, 2-, 3-, 4-, 5-, 6-, 7- or 8-ethyldecyl, 1-, 2-, 3-, 4-, 5-, 6-, 7- or 8-ethyldecyl, 1-, 2-, 3-, 4-, 5- or 6-propylnonyl, 1-, 2-, 3- or 4-butyloctyl, 1-2-pentylheptyl and the like. Illustrative of cyclic alkyl are cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl, cyclooctyl, cyclononyl and cyclodecyl and the like.

Examples of hydrophilic moieties include oligonucleotides up to 20 nucleotides in length, peptides up to 20 amino acids in length, peptide mimics, carbohydrates, oligosaccharides and derivatives thereof.

It will be appreciated by those skilled in the art that the prodrugs of formula (I) may be modified to provide pharmaceutically acceptable derivatives thereof at any one or more of the functional groups in the prodrugs of formula (I). Of particular interest as such derivatives are prodrugs modified at the carboxyl function, hydroxyl function or at amino groups. Thus, prodrugs of interest include alkyl esters, such as methyl, ethyl, propyl or isopropyl esters, aryl esters, such as phenyl, benzoyl esters, and acetyl esters of the prodrugs of formula (I).

The term "pharmaceutically acceptable derivative" means any pharmaceutically acceptable salt, ether, ester or salt of such ester of a prodrug of formula (I) or any other compound which, upon administration to the subject, is capable of providing a prodrug of formula (I) or a pharmaceutically active metabolite or residue thereof.

Pharmaceutically acceptable salts of the prodrugs of formula (I) include those derived from pharmaceutically acceptable inorganic and organic acids and bases. Examples of suitable acids include hydrochloric, hydrobromic, sulphuric, nitric, perchloric, fumaric, maleic, phosphoric, glycollic, lactic, salicylic, succinic, toluene-p-sulphonic, tartaric, acetic, citric, methanesulphonic, formic, benzoic, malonic, naphthalene-2-sulphonic and benzenesulphonic acids. Other acids such as oxalic acid, while not in themselves pharmaceutically acceptable, may be useful in the preparation of salts useful as intermediates

in obtaining prodrugs of the invention and their pharmaceutically acceptable acid addition salts.

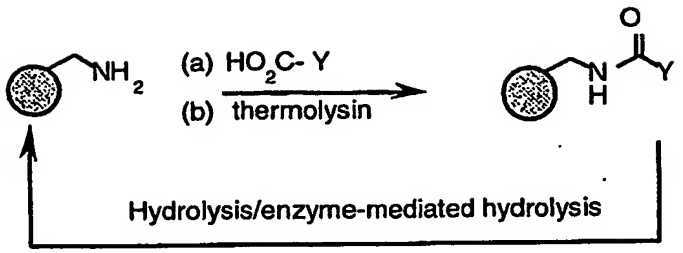
Salts derived from appropriate bases include alkali metal (*eg.* sodium), alkaline earth metal (*eg.* magnesium), ammonium, and NR_4^+ (where R is C_{1-4} alkyl) salts.

The prodrugs of the invention may be prepared by methods described herein. It will be apparent to those skilled in the art, that it may be necessary to use protecting groups to protect one or more functional groups of the pharmaceutically active moiety during the process of attaching the pharmaceutical moiety to the linker group and the pharmacokinetic regulator. See for example "Protective Groups in Organic Synthesis" by T.W. Green and P.G.M. Nuts (John Wiley & Sons, 1991).

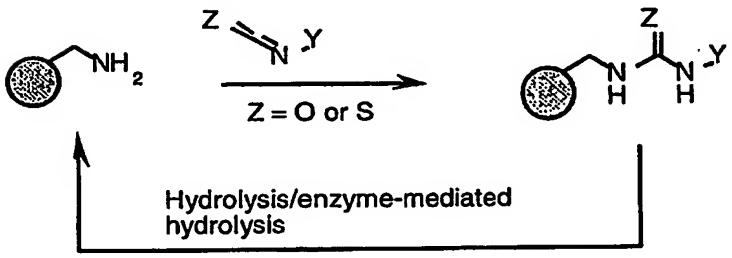
The chemistry of the linking reaction will be determined either by the nature of reactive functional groups present in the pharmaceutical moiety or the nature of reactive groups that can be introduced to the pharmaceutical moiety using a series of chemical transformations. General methods for preparing the prodrugs will now be described with reference to the nature of the functional group present in or introduced to the pharmaceutical moiety. It should be noted that many of the prodrugs described herein can be prepared using either conventional chemical methods or enzymatic methods. Enzymatic methods can in some instances provide greater selectivity than conventional methods. It will be appreciated that the invention is not limited to such methods.

<u>Pharmaceutical moieties bearing amines or to which amines can be readily introduced</u>

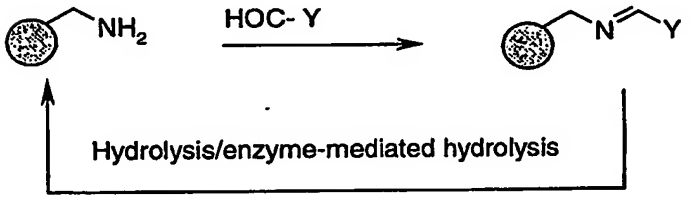
1) Amide formation
   a) Conventional chemical means
   b) Enzymatic peptide coupling using, for example, thermolysin



2) Urea or thiourea formation



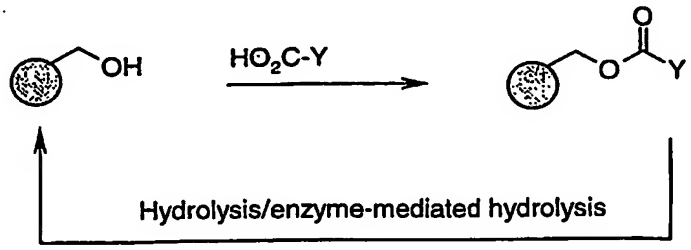
3) Imine formation



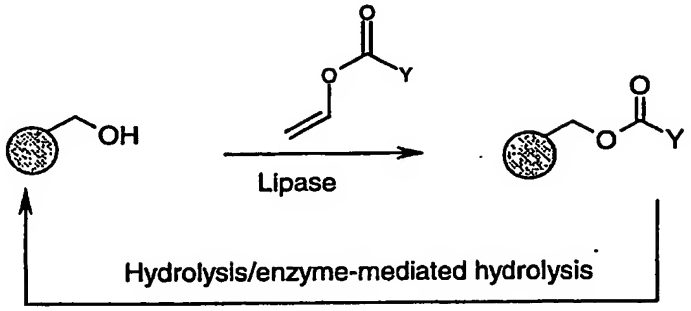
Pharmaceutical moieties bearing alcohols or to which alcohols can be readily introduced

1) Ester Formation

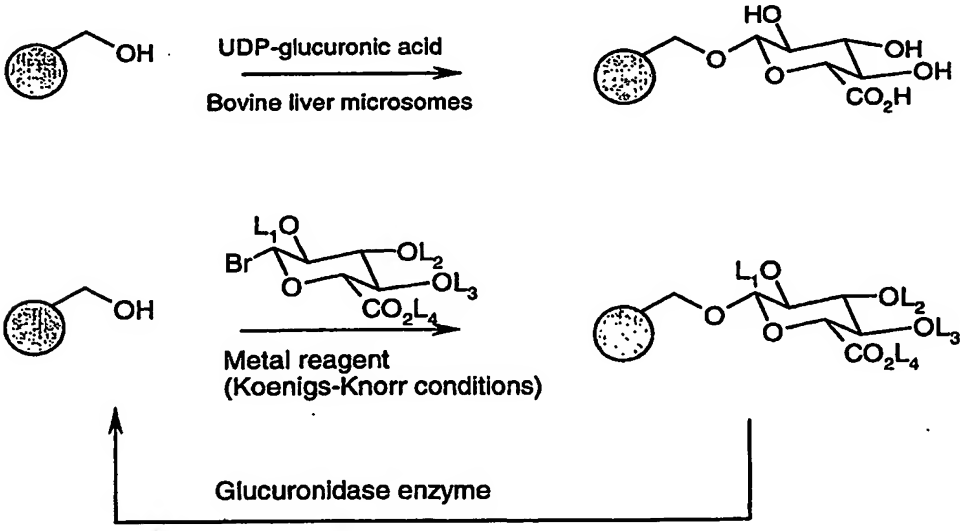
a) Conventional chemical means



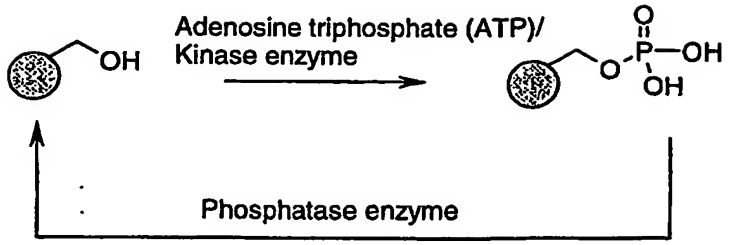
b) Transesterification using, for example, lipase enzymes



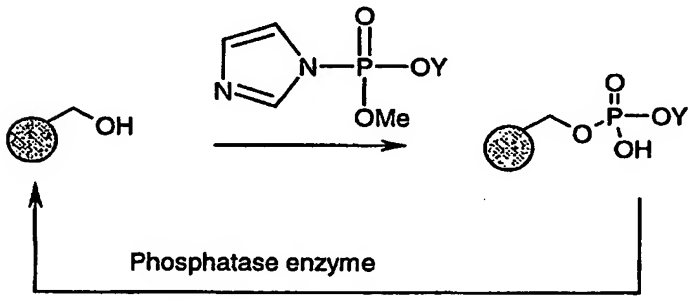
2) Acetal formation, for example, introduction of a glucuronic acid residue or a lipophile-modified version of glucuronic acid



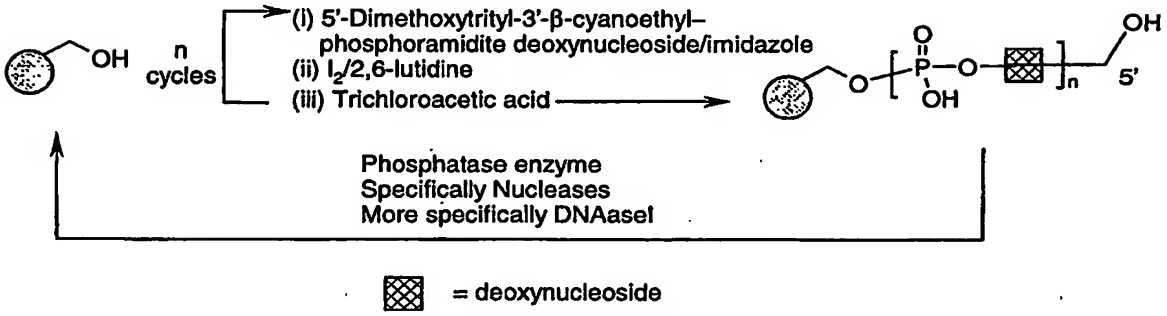
3) Phosphate formation



4) Phosphate diester formation



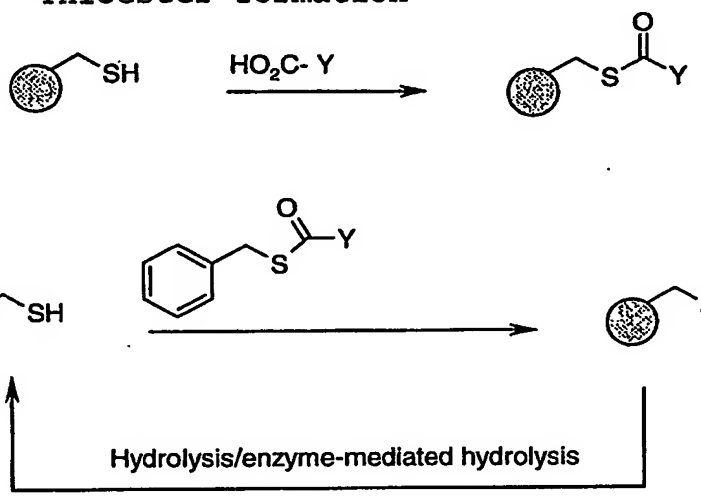
5) Oligonucleotide formation



Once formed, the drug-oligonucleotide conjugate can either be base-paired with a complementary oligonucleotide according to Watson-Crick or Hoogsteen base pairing principles or can be left single stranded. The choice of whether to use double stranded, triple stranded or single stranded DNA depends on the particular phosphatase that will be used for the conversion of the prodrug into the drug.

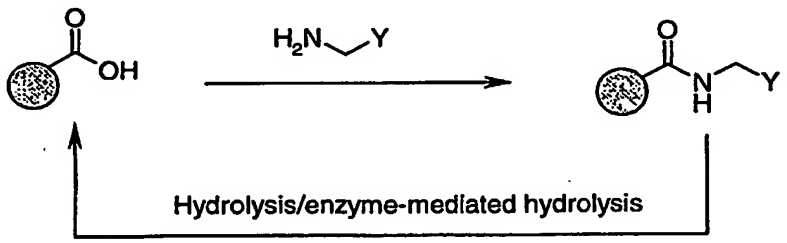
Pharmaceutical moieties bearing thiols or to which thiols can be readily introduced

1. Thioester formation

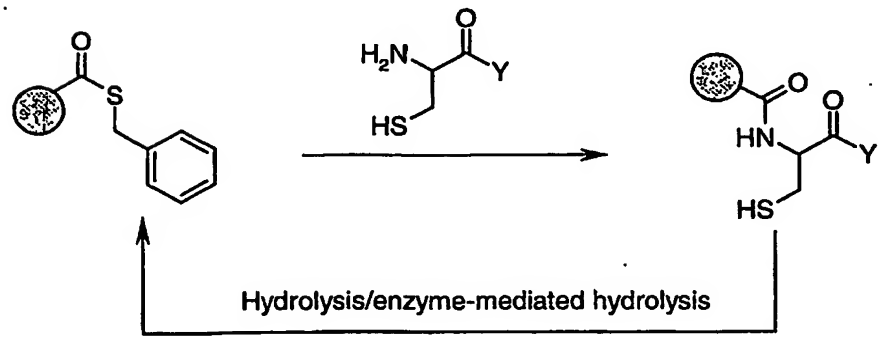


Pharmaceutical moieties bearing carboxylic acids or to which carboxylic acids can be readily introduced

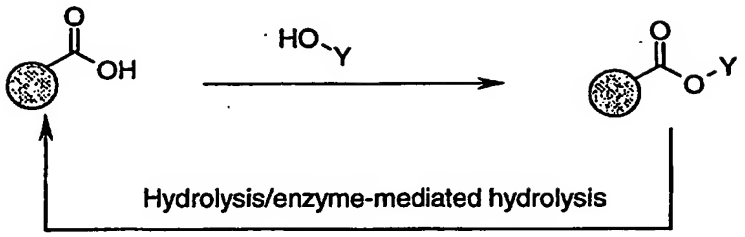
1. Amide formation



2. Amide formation (via thioesters)

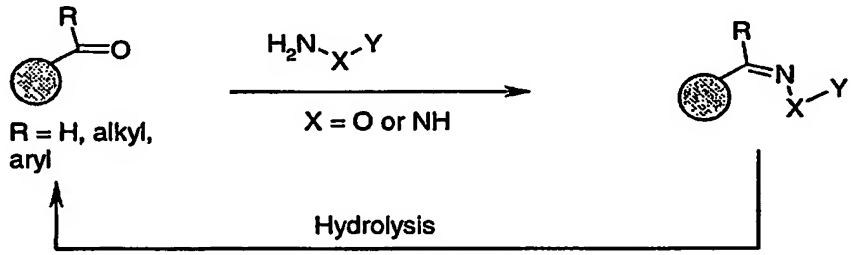


3. Ester formation



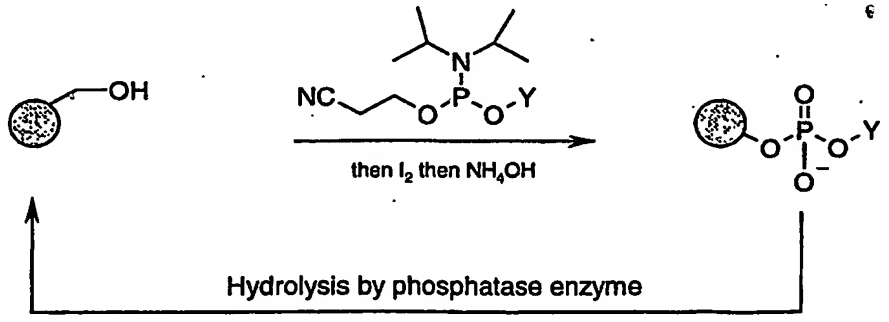
Pharmaceutical moieties bearing aldehydes and ketones or to which aldehydes and ketones can be readily introduced

1. Oxime or hydrazone formation



Pharmaceutical moieties bearing phosphate groups or their derivatives

1. Phosphate-linked dimers can be prepared as shown below.



When the pharmaceutical moiety is the preferred antimicrobial agent, then modification is preferably

carried out with the primary goal of increasing residence time but this may also be accompanied by an increase in potency or therapeutic index. The choice of position at which modification should be carried out should be guided by knowledge of how the antimicrobial agent is likely to be revealed by enzymes present in the subject or knowledge of the enzymes produced by the antimicrobial agent.

Pharmaceutically acceptable salts of the prodrugs of formula (I) may be prepared according to known procedures.

For use in therapy it is preferable that the prodrugs of formula (I) are in crystalline form.
The prodrugs of formula (I) depending on the nature of the pharmaceutically active moiety may possess antimicrobial activity, preferably antibacterial activity.

The term "microbial infection" is used herein in its broadest sense and refers to any infection caused by a microorganism and includes viral and bacterial infections. Examples of such infectious microorganisms may be found in a number of well known texts such as 'Medical Microbiology' (Greenwood, D., Slack, R., Peutherer, J., Churchill Livingstone Press, 2002); 'Mims' Pathogenesis of Infectious Disease' (Mims, C., Nash, A., Stephen, J., Academic Press, 2000); '"Fields" Virology. (Fields, B.N., Knipe, D.M., Howley, P.M., Lippincott Williams and Wilkins, 2001).

The term "microorganism" includes any microscopic organism or taxonomically related macroscopic organism within the categories algae, bacteria, fungi, protozoa, viruses and subviral agents or the like. Although, the preferable microorganism is those found in sources described above. For example, those microorganisms found in anaerobic sludge such as methanogens, eubacteria or nitrifying bacteria.

Viral infections include, but are not limited to those caused by Adenovirus, Lassa fever virus (Arenavirus), Astrovirus, Hantavirus, Rift Valley Fever virus (Phlebovirus), Calicivirus, Ebola virus, Marburg Virus, Japanese encephalitis virus, Dengue virus, Yellow fever

virus, Hepatitis C virus, Hepatitis G virus, Hepatitis B virus, Hepatitis D virus, Herpes simplex virus 1, Herpes simplex virus 2, Cytomegalovirus, Epstein Barr virus, Varicella Zoster Virus, Human Herpesvirus 7, Human Herpesvirus 8, Influenza virus, Parainfluenza virus, Rubella virus, Mumps virus, Morbillivirus, Measles virus, Respiratory Syncytial virus, Papillomaviruses, JC virus (Polyomavirus), BK virus (Polyomavirus), Parvovirus, Coxsackie virus (A and B), Hepatitis A virus, Polioviruses, Rhinoviruses, Reovirus, Rabies Virus (Lyssavirus), Human Immunodeficiency virus 1 and 2 and Human T-cell Leukemia virus.

Examples of viral infections include Adenovirus acute respiratory disease, Lassa fever, Astrovirus enteritis, Hantavirus pulmonary syndrome, Rift valley fever, Hepatitis E, diarrhoea, Ebola hemorrhagic fever, Marburg hemorrhagic fever, Japanese encephalitis, Dengue fever, Yellow fever, Hepatitis C, Hepatitis G, Hepatitis B, Hepatitis D, Cold sores, Genital sores, Cytomegalovirus infection, Mononucleosis, Chicken Pox, Shingles, Human Herpesvirus infection 7, Kaposi Sarcoma, Influenza, Brochiolitis, German measles, Mumps, Measles (rubeola), Measles, Brochiolitis, Papillomas (Warts), cervical cancer, Progressive multifocal leukoencephalopathy, Kidney disease, Erythema infectiosum, Viral myocarditis, meninigitis, entertitis, Hepititis, Poliomyelitis, Cold, Diarrhoea, Rabies, AIDS and Leukemia.

Preferably, the viral infection is an orthomyxovirus or paramyxovirus infection, for example, influenza A or B, parainfluenza, mumps or Newcastle disease. More preferably the viral infection is an influenza A or B infection.

Bacterial infections include, but are not limited to, infections caused by Gram Positive Bacteria including *Bacillus cereus, Bacillus anthracis, Clostridium botulinum, Clostridium difficile, Clostridium tetani, Clostridium perfringens, Corynebacteria diphtheriae, Enterococcus (Streptococcus D), Listeria monocytogenes, Pneumoccoccal infections (Streptococcus pneumoniae), Staphylococcal*

infections and Streptococcal infections ; Gram Negative Bacteria including *Bacteroides, Bordetella pertussis, Brucella, Campylobacter* infections, enterohaemorrhagic *Escherichia coli* (EHEC/*E. coli* 0157 : H7) enteroinvasive *Escherichia coli* (EIEC), enterotoxigenic *Escherichia coli* (ETEC), *Haemophilus influenzae, Helicobacter pylori, Klebsiella pneumoniae, Legionella* spp., *Moraxella catarrhalis, Neisseria gonnorrhoeae, Neisseria meningitidis, Proteus* spp., *Pseudomonas aeruginosa, Salmonella* spp., *Shigella* spp., *Vibrio cholera* and *Yersinia*; acid fast bacteria including *Mycobacterium tuberculosis, Mycobacterium avium-intracellulare, Myobacterium johnei, Mycobacterium leprae*, atypical bacteria, *Chlamydia, Mycoplasma, Rickettsia, Spirochetes, Treponema pallidum, Borrelia recurrentis, Borrelia burgdorfii* and *Leptospira icterohemorrhagiae*; or other miscellaneous bacteria, including *Actinomyces* and *Nocardia*.

Preferably, the bacterial infection is a Gram Negative or Gram Positive infection such as infections associated with the respiratory tract (e.g. pneumonia associated with Klebsiella, mycobacterium species including tuberculosis and pseudomonas aeruginosa), urinary tract and systemic infections caused by enteric bacteria, GI tract diseases such as Shigella dysentery and plague.

Fungal infections include, but are not limited to, infections caused by *Alternaria alternata, Aspergillus flavus, Aspergillus fumigatus, Aspergillus nidulans, Aspergillus niger, Aspergillus versicolor, Blastomyces dermatiditis, Candida albicans, Candida dubliensis, Candida krusei, Candida parapsilosis, Candida tropicalis, Candida glabrata, Coccidioides immitis, Cryptococcus neoformans, Epidermophyton floccosum, Histoplasma capsulatum, Malassezia furfur, Microsporum canis, Mucor* spp., *Paracoccidioides brasiliensis, Penicillium marneffei, Pityrosporum ovale, Pneumocystis carinii, Sporothrix schenkii, Trichophyton rubrum, Trichophyton interdigitale, Trichosporon beigelii* and *Rhodotorula* spp..

Yeast infections include, but are not limited to, infections caused by *Brettanomyces clausenii, Brettanomyces custerii, Brettanomyces anomalous, Brettanomyces naardenensis, Candida himilis, Candida intermedia, Candida saki, Candida solani, Candida tropicalis, Candida versatilis, Candida bechii, Candida famata, Candida lipolytica, Candida stellata, Candida vini, Debaromyces hansenii, Dekkera intermedia, Dekkera bruxellensis, Geotrichium sandidum, Hansenula fabiani, Hanseniaspora uvarum, Hansenula anomala, Hanseniaspora guillermondii Hanseniaspora vinae, Kluyveromyces lactis, Kloekera apiculata, Kluveromyces marxianus, Kluyveromyces fragilis, Metschikowia pulcherrima, Pichia guilliermodii, Pichia orientalis, Pichia fermentans, Pichia memranefaciens, Rhodotorula Saccharomyces bayanus, Saccharomyces cerevisiae, Saccharomyces dairiensis Saccharomyces exigus, Saccharomyces uinsporus, Saccharomyces uvarum, Saccharomyces oleaginosus, Saccharomyces boulardii, Saccharomycodies ludwigii, Schizosaccharomyces pombe, Torulaspora delbruekii, Torulopsis stellata, Zygoaccharomyces bailli* and *Zygosaccharomyces rouxii*.

Protozoal infections include, but are not limited to, infections caused by Leishmania, Toxoplasma, Plasmodia, Theileria, Anaplasma, Giardia, Trichomonas, Trypanosoma, Coccidia, and Babesia. Specific examples include *Trypanosoma cruzi, Eimeria tenella, Plasmodium falciparum, Plasmodium vivax* or *Plasmodium ovale*.

Preferably the subject is an animal such as a mammal, more preferably a human, or a member of the genus Equus, for example a horse, donkey or mule. Most preferably the mammal is a human.

As used herein, the term "effective amount" is meant an amount of the compound of formula (I) effective to preventing or treating a microbial infection in order to yield a desired therapeutic response. For example, to overcome or alleviate the effects of a microbial infection.

The term "therapeutically-effective amount" means an amount of the prodrug of formula (I) to yield a desired therapeutic response. For example, treating or preventing a microbial infection.

The specific "therapeutically-effective amount" will, obviously, vary with such factors as the particular microbial infection being treated, the physical condition of the subject, the type of animal being treated, the duration of the treatment, the nature of concurrent therapy (if any), and the specific formulation employed and the structure of the compound or its derivatives.

Generally, the terms "treating", "treatment" and the like are used herein to mean affecting a subject, tissue or cell to obtain a desired pharmacologic and/or physiologic effect. The effect may be prophylactic in terms of completely or partially preventing a microbial infection or sign or symptom thereof, and/or may be therapeutic in terms of a partial or complete cure of a microbial infection. "Treating" as used herein covers any treatment of, or prevention of a microbial infection in a vertebrate, a mammal, particularly a human, and includes: (a) preventing the microbial infection from occurring in a subject that may be predisposed to the microbial infection, but has not yet been diagnosed with the microbial infection; (b) inhibiting the microbial infection, i.e., arresting its development; or (c) relieving or ameliorating the effects, i.e., cause regression of the symptoms of the microbial infection.

The prodrugs of the invention may also be used in diagnostic methods, in particular methods for the detection of microbial infections such as the influenza virus. For use in such methods it may be advantageous to link a prodrug of the invention to a label, such as a radioactive, fluorescent or chemiluminescent label.

Methods of diagnosis for which the prodrugs of the invention are suitable are described, for example, in our earlier applications PCT/AU97/00109 and PCT/AU97/00771.

It will be further appreciated that the amount of the prodrug of the invention required for use in treatment will vary not only with the particular prodrug selected but also with the route of administration, the nature of the condition being treated, and the age and condition of the subject, and will ultimately be at the discretion of the attendant physician or veterinarian. In general however, a suitable dose will be in the range of from about 0.001 to 100 mg/kg of bodyweight per day, preferably in the range of 0.001 to 1 mg/kg/day, most preferably in the range of 0.002 to 0.1 mg/kg/day.

Treatment is preferably commenced before or at the time of infection and continued until microorganism is no longer present. However the prodrugs are also effective when given post-infection, for example, after the appearance of established symptoms.

Suitably treatment is given on one or two occasions, preferably only once only for treatment and preferably once per week for prophylaxis.

The prodrug is conveniently administered in unit dosage form, for example containing 1 to 100 mg, more conveniently 0.1 to 10 mg, most conveniently 0.1 to 5 mg of active ingredient per unit dosage form.

While it is possible that, for use in therapy, the prodrug of the invention may be administered as the raw chemical, it is preferable to present the active ingredient as a pharmaceutical formulation.

The carrier(s) must be "acceptable" in the sense of being compatible with the other ingredients of the formulation and not being deleterious to the subject thereof.

The prodrugs of the invention may also be used in combination with other therapeutic and/or prophylactic agents, for example other antimicrobial or antiinfective agents. In particular the prodrugs of the invention may be employed with other antibacterial agents.

The combinations referred to above may conveniently be presented for use in the form of a pharmaceutical formulation and thus such formulations

comprising a combination as defined above together with a pharmaceutically acceptable carrier form part of the invention.

Suitable therapeutic and/or prophylactic agents for use in such combinations include other antimicrobial agents, in particular antibacterial agents such as combinations of trimethoprim and sulfonamide; bacitracin and polymyxin B-neomycin; imipenem and fluoroquinolone; and beta-lactam and aminoglycoside.

The individual components of such combinations may be administered either separately, sequentially or simultaneously in separate or combined pharmaceutical formulations.

When the prodrugs of the invention are used with a second therapeutic and/or prophylactic agent active against the same microorganism, the dose of each prodrug may either be the same as or different from that employed when each prodrug is used alone. Appropriate doses will be readily appreciated by those skilled in the art.

Pharmaceutical formulations include those suitable for oral, rectal, nasal, topical (including buccal and sub-lingual), vaginal or parenteral (including intramuscular, sub-cutaneous and intravenous) administration, or those in a form suitable for administration to the respiratory tract (including the nasal passages) for example by inhalation or insufflation. The formulations may, where appropriate, be conveniently presented in discrete dosage units, and may be prepared by any of the methods well known in the art of pharmacy. These methods include the step of bringing into association the prodrug with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product into the desired formulation.

Pharmaceutical formulations suitable for oral administration may conveniently be presented as discrete units such as capsules, cachets or tablets each containing a predetermined amount of the prodrug; as a powder or granules; as a solution, a suspension or as an emulsion.

The prodrug may also be presented as a bolus, electuary or paste. Tablets and capsules for oral administration may contain conventional excipients such as binding agents, fillers, lubricants, disintegrants, or wetting agents. The tablets may be coated according to methods well known in the art. Oral liquid preparations may for example be in the form of aqueous or oily suspensions, solutions, emulsions, syrups or elixirs, or may be presented as a dry product for constitution with water or other suitable vehicle before use. Such liquid preparations may contain conventional additives such as suspending agents, emulsifying agents, non-aqueous vehicles, which may include edible oils, or preservatives.

The prodrugs according to the invention may also be formulated for parenteral administration by injection, for example bolus injection, or continuous infusion, and may be presented in unit dose form in ampoules, pre-filled syringes, small volume infusion or in multi-dose containers with an added preservative. The compositions may take such forms as suspensions, solutions, or emulsions in oily or aqueous vehicles, and may contain formulating agents such as suspending, stabilising and/or dispersing agents. Alternatively, the prodrug may be in powder form, obtained by aseptic isolation of sterile solid or by lyophilisation from solution, for constitution with a suitable vehicle, *eg.* sterile, pyrogen-free water, before use.

For topical administration to the epidermis the prodrugs according to the invention may be formulated as ointments, creams or lotions, or as a transdermal patch. Ointments and creams may, for example, be formulated with an aqueous or oily base with the addition of suitable thickening and/or gelling agents. Lotions may be formulated with an aqueous or oily base, and will in general also contain one or more emulsifying agents, stabilising agents, dispersing agents, suspending agents, thickening agents, or colouring agents.

Formulations suitable for topical administration in the mouth include lozenges comprising the prodrug in a flavoured base, usually sucrose and gum acacia or gum

tragacanth; pastilles comprising the prodrug in an inert base such as gelatin or sucrose and gum acacia; and mouthwashes comprising the prodrug in a suitable liquid carrier.

Pharmaceutical formulations suitable for rectal administration wherein the carrier is a solid are most preferably presented as unit dose suppositories. Suitable carriers include cocoa butter and other materials commonly used in the art, and the suppositories may be conveniently formed by admixture of the prodrug with the softened or melted carrier(s) followed by chilling and shaping moulds.

Formulations suitable for vaginal administration may be presented as pessaries, tampons, creams, gels, pastes, foams or sprays containing in addition to the active ingredient such carriers as are known in the art to be appropriate.

For administration to the respiratory tract, including intranasal administration, the neuraminidase inhibitors may be administered by any of the methods and formulations employed in the art for administration to the respiratory tract.

Thus in general the prodrugs may be administered in the form of a solution or a suspension or as a dry powder.

Solutions and suspensions will generally be aqueous, for example prepared from water alone (for example sterile or pyrogen-free water) or water and a physiologically acceptable co-solvent (for example ethanol, propylene glycol or polyethylene glycols such as PEG 400).

Such solutions or suspensions may additionally contain other excipients for example preservatives (such as benzalkonium chloride), solubilising agents/surfactants such as polysorbates (*eg.* Tween 80, Span 80, benzalkonium chloride), buffering agents, isotonicity-adjusting agents (for example sodium chloride), absorption enhancers and viscosity enhancers. Suspensions may additionally contain suspending agents (for example microcrystalline cellulose, carboxymethyl cellulose sodium).

Solutions or suspensions are applied directly to the nasal cavity by conventional means, for example with a dropper, pipette or spray. The formulations may be provided in single or multidose form. In the latter case a means of dose metering is desirably provided. In the case of a dropper or pipette this may be achieved by the subject administering an appropriate, predetermined volume of the solution or suspension. In the case of a spray this may be achieved for example by means of a metering atomising spray pump.

Administration to the respiratory tract may also be achieved by means of an aerosol formulation in which the compound is provided in a pressurised pack with a suitable propellant, such as a chlorofluorocarbon (CFC), for example dichlorodifluoromethane, trichlorofluoromethane or dichlorotetrafluoroethane, carbon dioxide or other suitable gas. The aerosol may conveniently also contain a surfactant such as lecithin. The dose of drug may be controlled by provision of a metered valve.

Alternatively the prodrugs may be provided in the form of a dry powder, for example a powder mix of the compound in a suitable powder base such as lactose, starch, starch derivatives such as hydroxypropylmethyl cellulose and polyvinylpyrrolidine (PVP). Conveniently the powder carrier will form a gel in the nasal cavity. The powder composition may be presented in unit dose form, for example in capsules or cartridges of *eg.* gelatin, or blister packs from which the powder may be administered by means of an inhaler.

In formulations intended for administration to the respiratory tract, including intranasal formulations, the prodrug will generally have a small particle size, for example of the order of 5 microns or less. Such a particle size may be obtained by means known in the art, for example by micronisation.

When desired, formulations adapted to give sustained release of the prodrug may be employed.

Preferably the prodrugs of the invention are administered to the respiratory tract by inhalation,

insufflation or intranasal administration, or a combination thereof.

"Relenza" is administered by oral inhalation as a free-flow powder via a "Diskhaler" (trade mark of Glaxo Wellcome plc). A similar formulation would be suitable for the present invention.

It will be appreciated that the inhaler may also be in the form of a meter dose aerosol inhaler.

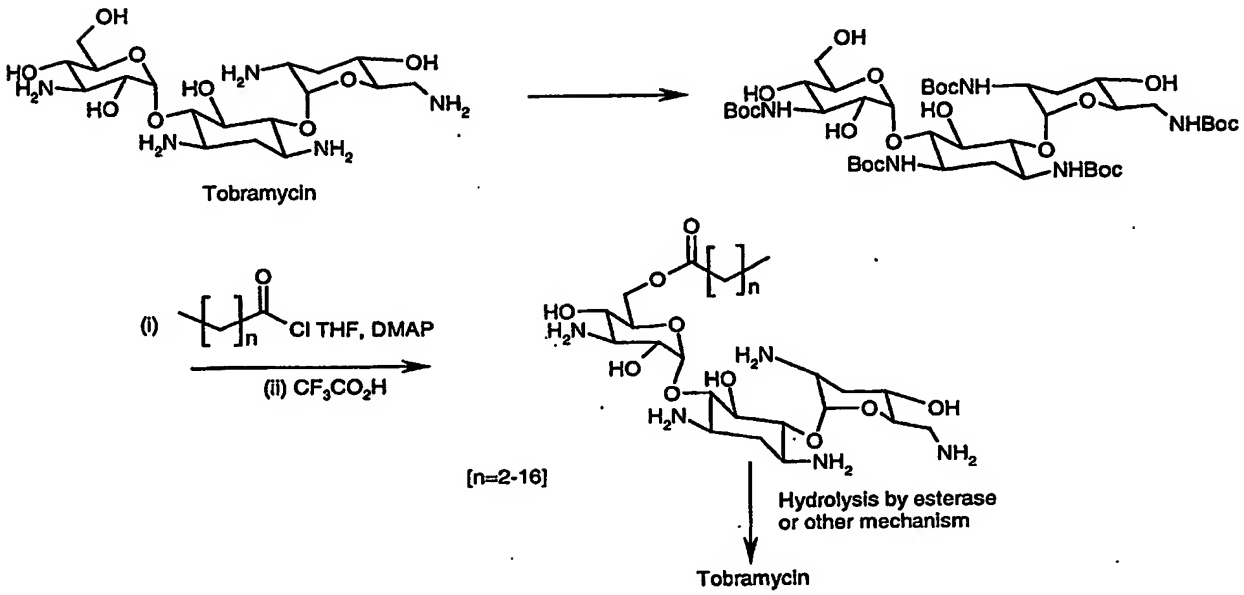
All publications, including but not limited to patents and patent applications, cited in this specification are herein incorporated by reference as if each individual publication were specifically and individually indicated to be incorporated by reference herein as though fully set forth.

EXAMPLES

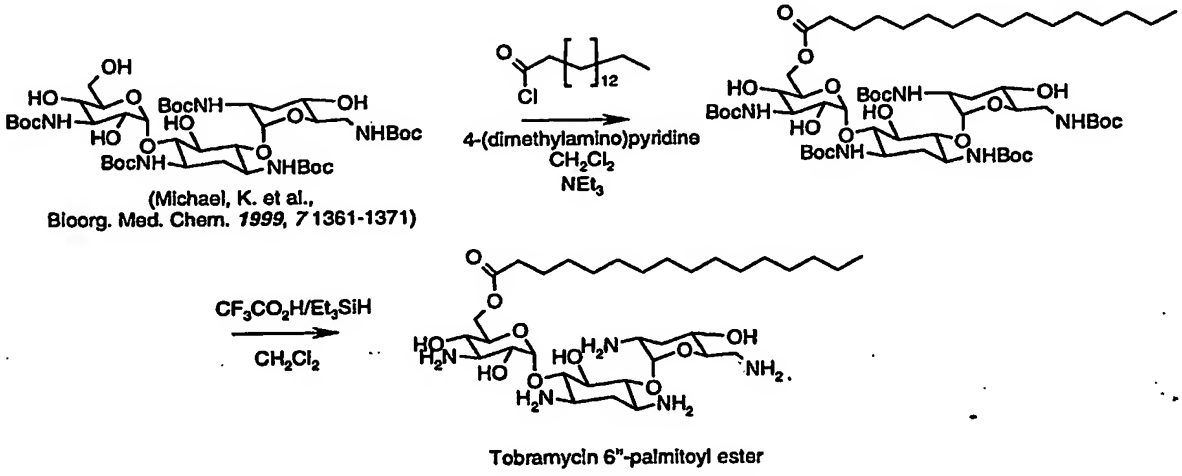
The invention will now be described in detail by way of reference only to the following non-limiting examples.

The examples describe methods for the preparation of hydrolysis-activated prodrugs of aminoglycoside antibiotic tobramycin.

Example 1: Ester linkage - activation by hydrolysis



Example 2: Tobramycin 6''-palmitoyl ester

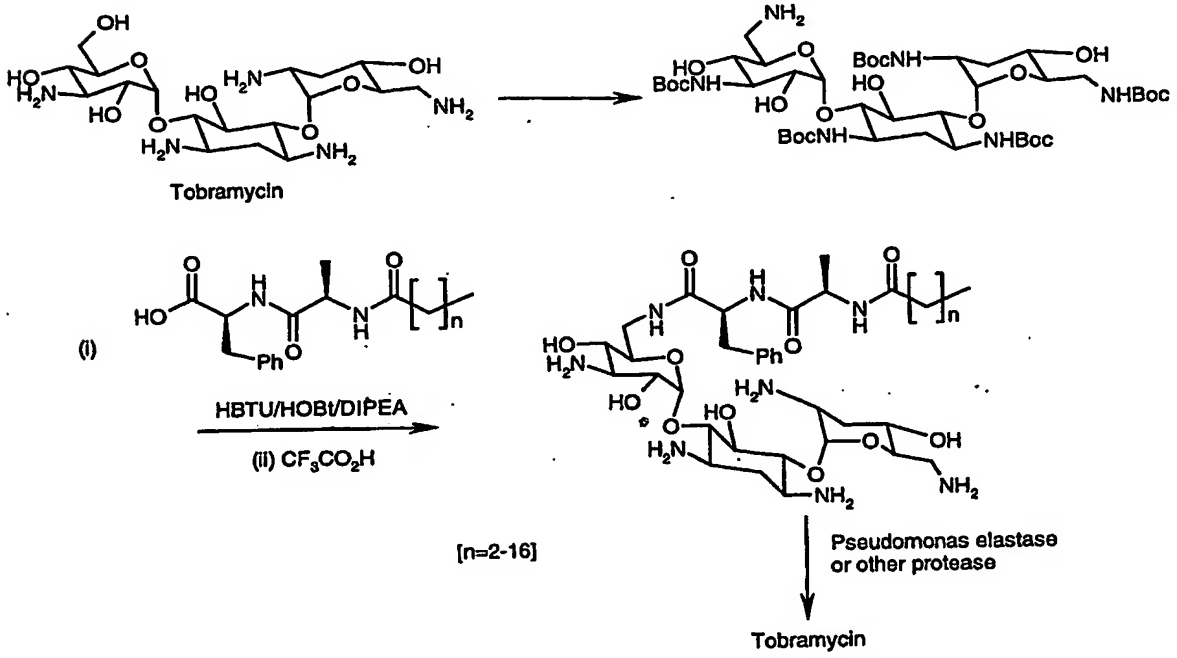


Boc-tobramycin (68mg, 0.07mmole) in dichloromethane (15mL) was treated with 4-(dimethylamino)pyridine (13.2mg, 0.108mmole) and palmitoyl chloride (27.5mg, 0.1mmole). The reaction mixture was

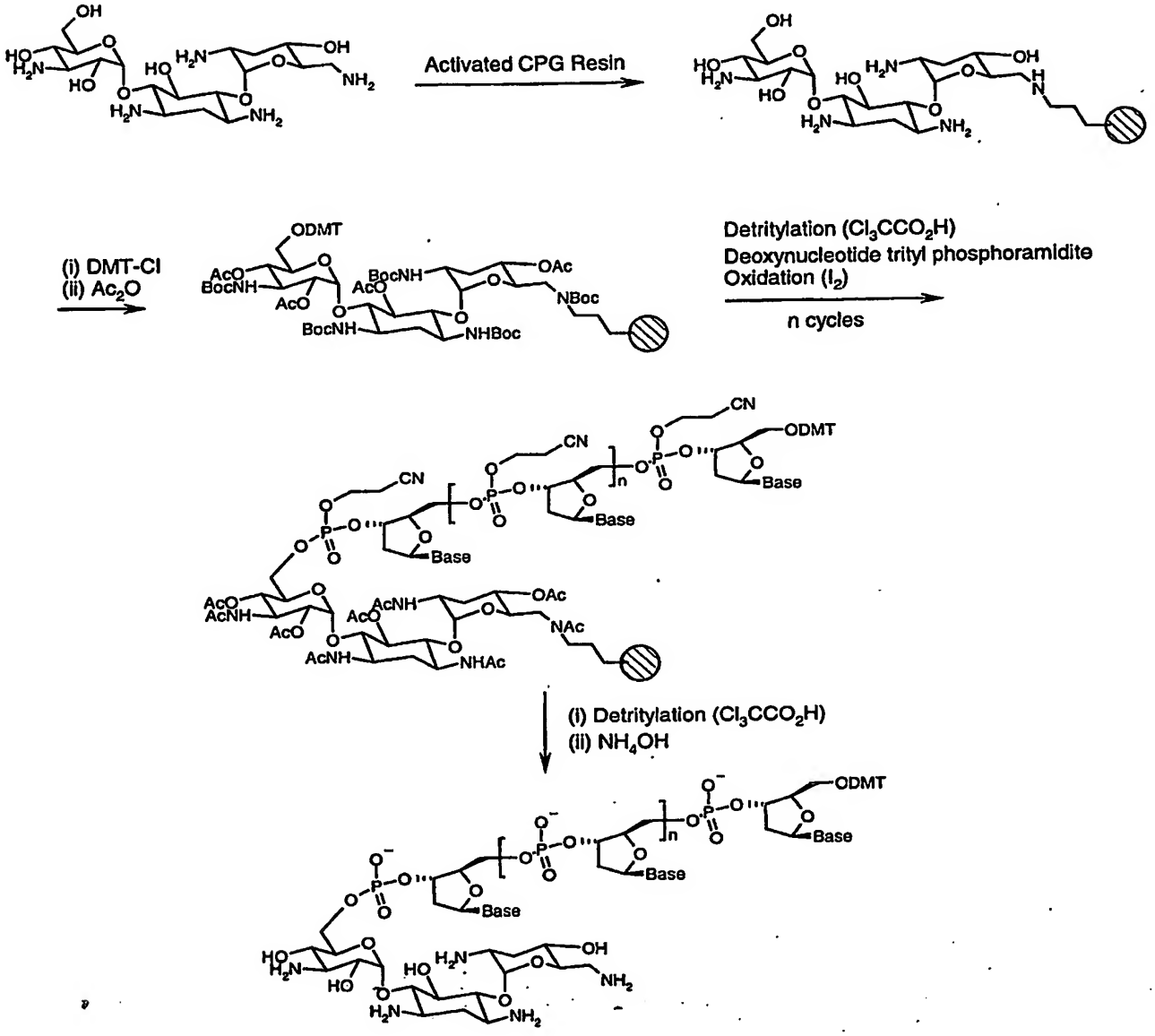
stirred at room temperature for 30 minutes then treated with triethylamine (143µL, 1.08mmole) and stirred for a further 18 hours at room temperature. The dichloromethane was then removed under reduced pressure and the residue dissolved in 1:1 water:diethyl ether. The ether layer was washed with water and saturated aqueous sodium bicarbonate then dried, filtered and concentrated to give 61mg of residue.

The residue was dissolved in dichloromethane (3mL) and then treated with triethylsilane (100µL) and trifluoroacetic acid (3mL). After 2 hours stirring at room temperature, the volatiles were removed under reduced pressure and the residue purified by preparative LCMS. Calculated for $C_{34}H_{67}N_5O_{10}$, 705. Found (ESMS) 706 ([M+H]+).

Example 3: Amide linkage - activation by hydrolysis



Example 4: Oligodeoxynucleotide formation



Example 5: Assessment of long duration of action

Rodents were anaesthetised with Ketamine/Domitor mixture according to standard procedures and dosed with compound of interest by the intra-nasal route at a dose volume of approximately 3.0ml/kg. The rodent is held in the vertical position during dosing of 30µL per nostril. At different time points, for example, 2, 8, 24, 48 and 168

hours post-dose, levels of compound in the lung tissue are assessed by analytical methods. Any analytical method suitable for detection of this type of compound may be used. The time at which levels of compound fall below the sensitivity of the analytical techniques identified will determine the residency time of the compound in lung tissue.

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2)

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It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.